A MICROMETHOD FOR THE DETERMINATION OF SODIUM

By ANCEL P. WEINBACH

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In view of the increasing importance of acid-base equilibrium and of the rôle of the inorganic elements in the body economy, the accurate determination of sodium in the blood and body fluids becomes of increasing importance. Barber and Kolthoff (1) in 1928 were the first to employ the triple salt, uranyl zinc sodium acetate, for the quantitative determination of sodium. They ascribed to this salt the composition $(UO_2)_3ZnNa(CH_3COO)_9 \cdot 6H_2O$, the amount of sodium in a weighed precipitate of this salt being 0.01495 the weight of the precipitate.

Butler and Tuthill (2) applied the gravimetric procedure of Barber and Kolthoff for the determination of sodium in biological material. For urine, they first precipitated the phosphate (which interferes with the gravimetric method by precipitating as insoluble uranyl phosphate) with calcium hydroxide, and they precipitated the protein with solid mercuric chloride. In their determination of sodium in blood serum they used 1 cc., digesting it first as in a micro-Kjeldahl method before proceeding with the gravimetric determination.

These gravimetric methods give fairly good results, but the methods are tedious and require relatively large amounts of material. Salit (3) describes a modified procedure for the gravimetric determination of sodium in urine and feces, and a modified colorimetric method for whole blood or serum, in which but 0.5 cc. of serum is required or 0.2 cc. of whole blood. Salit's modification

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consisted of precipitating the triple salt, uranyl zinc sodium acetate, by the addition of alcohol, rather than by having the reagent saturated with the triple salt as in the original Barber-Kolthoff method. Salit's colorimetric method is based on the red color which uranium salts give with potassium ferrocyanide.

Using Salit's modification of precipitating the uranyl zinc sodium acetate by the addition of alcohol, we have developed a titrimetric method for the determination of sodium in 0.1 cc. of blood from the finger tip or 0.5 cc. of a 1:5 trichloroacetic acid protein-free serum or plasma filtrate or 1 cc. of a 1:10 trichloroacetic acid protein-free whole blood or cell filtrate.

Principle

The sodium is precipitated in alcoholic medium as the triple salt, uranyl zinc sodium acetate. Subsequently the salt is titrated with sodium hydroxide, with phenolphthalein as an indicator, the uranium and zinc forming amphoteric hydroxides, as follows:

$$(UO_2)_3ZnNa(CH_3COO)_9 + 8NaOH \rightarrow 3(UO_2)(OH)_2 + Zn(OH)_2 + 9Na(CH_3COO)$$

Procedure

For 0.1 Cc. of Serum or Whole Blood—With an accurate 0.1 cc. pipette collect 0.1 cc. of serum or whole blood and transfer it to 1.5 cc. of water in a 15 cc. centrifuge tube. From a 1 cc. graduated pipette add, with mixing, exactly 0.4 cc. of 20 per cent trichloroacetic acid. Centrifuge and transfer 1 cc. of the clear supernatant fluid to another 15 cc. centrifuge tube. Add 5 cc. of the uranyl zinc acetate reagent. From a 1 cc. graduated pipette add 0.3 cc. of 95 per cent alcohol and let stand for 5 minutes. Again add 0.3 cc. of alcohol and let stand for a few minutes. This procedure is repeated, without greatly disturbing the precipitate, until 2.1 cc. of alcohol have been added, the entire process of precipitation taking about ½ hour. Centrifuge, decant, drain on a pad of filter paper, and wipe the mouth of the tube with a cloth. Wash the precipitate once by blowing in 10 cc. of acetone wash reagent; centrifuge, decant, drain on a pad of filter paper, and wipe the mouth of the tube.

The precipitate, which is readily soluble in water, is then transferred quantitatively to a 100 cc. Erlenmeyer flask by blowing in

three or four 5 cc. portions of water. Add approximately 50 cc. of water and 0.5 cc. of 1 per cent phenolphthalein solution and titrate with 0.02 N NaOH to a just barely perceptible pink, with a microburette graduated in 0.02 cc.

A blank should be run to determine the amount of 0.02 N NaOH which will just give the end-point with distilled water.

If sufficient serum, plasma, whole blood, or cells is available, protein-free filtrates may be prepared on which sodium, potassium, magnesium, and phosphorus may be determined. These filtrates are prepared as follows:

Serum or Plasma—1 volume of serum or plasma is added to 3 volumes of water in a small flask. Add with shaking 1 volume of 20 per cent trichloroacetic acid, mix well, let stand 10 minutes, and filter through ashless filter paper.

Whole Blood or Cells—1 volume of whole blood or cells is added to 7 volumes of water in a small flask. Shake and let stand a few minutes till hemolysis is complete. Add rapidly, with shaking, 2 volumes of 20 per cent trichloroacetic acid. Mix well, let stand for 10 minutes, and filter through ashless filter paper.

Determine sodium, as described, on 0.5 cc. of the serum or plasma filtrate, 1 cc. of whole blood filtrate, and 1 or 2 cc. of cell filtrate. Determine sodium on 0.5 cc. of standard sodium solution to facilitate determining the end-point.

Calculation—From the equation for the reaction of the uranyl zinc sodium acetate with sodium hydroxide it is seen that $Na \simeq 8NaOH$. Then the weight of sodium in the sample taken would be given by the equation: Na in sample = (equivalents of NaOH) (23/8) × 1000 = (cc. of 0.02 N NaOH required − cc. of 0.02 N NaOH for blank) (0.00002) (23/8) × 1000 mg.

If 0.1 cc. of serum or plasma was used, the mg. of sodium found times 2000 would give mg. of sodium per 100 cc.; the equation reduces to: (actual cc. of 0.02 N NaOH) × 115 = mg. of sodium per 100 cc.

If 0.5 cc. of the 1:5 filtrate or 1 cc. of the 1:10 filtrate was used, the mg. of sodium found times 1000 would give mg. of sodium per 100 cc.; the equation reduces to: (actual cc. of 0.02 N NaOH) × 57.5 = mg. of sodium per 100 cc.

Reagents

1. Uranyl zinc acetate reagent. Solution A: 77 gm. of uranyl

acetate, $UO_2(CH_3COO)_2 \cdot 2H_2O$, and 14 cc. of glacial acetic acid are dissolved by gentle heating and stirring in about 400 cc. of water and diluted to 500 cc. in a volumetric flask. Solution B: 231 gm. of zinc acetate, $Zn(CH_3COO)_2 \cdot 3H_2O$, and 7 cc. of glacial acetic acid are dissolved by gentle heating and stirring in about 400 cc. of water and diluted to 500 cc. in a volumetric flask. The two solutions are mixed while hot, allowed to stand 24 hours or longer, and filtered.

2. Acetone wash reagent. A small amount of the triple salt, uranyl zinc sodium acetate, is prepared by adding 15 cc. of the uranyl zinc acetate reagent to 1 cc. of an approximately 5 per cent solution of sodium chloride with subsequent addition of about

TABLE I

Results on Known Solutions

Experiment No.	Na in unknown solutions	Na content determined
	mg. per cent	*mg. per cent*
1	393	393
2	Distilled water	0
3	157.2	156.8
4	235.8	236.1
5	864.6	864.0
6	786.0	785.0
7	Distilled water + potassium phosphate (no Na)	3.0

5 cc. of 95 per cent alcohol in small portions. Filter with suction and wash the precipitate with four or five small portions of 95 per cent alcohol and then with four or five small portions of ether, sucking dry after each addition of alcohol or ether. Add this amount of triple salt to a liter of acetone, shake, let stand overnight, and filter.

3. Standard sodium solution. Exactly 1 gm. of sodium chloride is dissolved in water and made up to a liter in a volumetric flask. Each cc. of this solution contains 0.393 mg. of sodium and 0.5 cc. is equivalent to exactly 3.42 cc. of 0.02 N NaOH.

DISCUSSION

The method described is applicable to the determination of sodium in blood, urine, or feces. Even relatively large amounts

of phosphate as are found in urine do not interfere with the determination, although the phosphate precipitates the uranium from the reagent as uranyl phosphate. The uranyl phosphate is insoluble in water and in the subsequent titration with sodium hydroxide there is no reaction with the uranium which has been precipitated as the phosphate. In determining the sodium content of urine, the urine is first wet ashed with nitric acid on the water bath or in an oven at a temperature of about 98°; for feces, dry ashing in a muffle is necessary.

As the reaction of sodium hydroxide and uranyl zinc sodium acetate is entirely stoichiometric, all ranges of sodium content may be determined by this method. Unknown solutions of sodium content known only to a disinterested third party were made up, and the sodium content determined. Table I gives the known sodium content of the solutions and the results as found with this method.

SUMMARY

A micromethod for the determination of sodium in 0.1 cc. of serum, plasma, or whole blood, or 0.5 cc. of a 1:5 trichloroacetic acid serum or plasma protein-free filtrate, or 1 cc. of a 1:10 trichloroacetic acid whole blood or cell protein-free filtrate, is described. The method may be adapted to the determination of sodium on other biological material, such as urine or feces.

The method is based on the precipitation of sodium in alcoholic medium, as the triple salt, uranyl zinc sodium acetate; subsequently, the precipitate, which is entirely soluble in water, is titrated with sodium hydroxide, the reaction depending on the formation of the amphoteric hydroxides of uranium and zinc.

BIBLIOGRAPHY

1. Barber, H. H., and Kolthoff, I. M., *J. Am. Chem. Soc.*, **50**, 1625 (1928); **51**, 3233 (1929).
2. Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, **93**, 171 (1931).
3. Salit, P. W., *J. Biol. Chem.*, **96**, 659 (1932).

V I T A

Ancel Paves Weinbach was born March 15, 1911, at Columbia, Missouri and attended the public schools of Columbia 1916–1927 and the University of Missouri thereafter, with the degrees of B. S. in Engineering, 1931, and A. M. in Biochemistry, 1933. He was assistant in Biochemistry in the School of Hygiene and Public Health of the Johns Hopkins University, 1934–1935.

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